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OFFICE OF PROJECTS AND GRANTS

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May 13, 1997
OPG:4731

Scientific Officer: 1141SB
Igor Vodyanoy
Office of Naval Research
800 North Quincy Street
Arlington, VA 22217-5000

Re: Grant No. N00014-90-J-1865

Dear Mr. Vodyanoy:

We enclose three (3) copies of the Final Technical Report for the referenced grant entitled "Electrical and Chemical Modulation of Synaptic Efficacy."

The report has been prepared by Dr. Mu-ming Poo, formerly of the University's Department of Biological Sciences, who served as the principal investigator.

We are pleased to submit the report for your consideration.

Sincerely yours,

Teri Crane
Senior Projects Officer

encl.
TC:tc

cc: Roger G. Swenson, Jr. - w/cy and Final Patent Report
Office of Naval Research
Boston Regional Office
495 Summer Street, Room 103
Boston, MA 02210-2109

Director, Naval Research Laboratory - w/cy
Attn: Code 2627
Washington, DC 20375

Defense Technical Information Center - w/ 2 cys ✔
Building 5, Cameron Station
Alexandria, VA ~~22304-6145~~ 22314

FINAL REPORT

Grant #: N00014-90-J-1865 R & T Code: 3414029

PRINCIPAL INVESTIGATOR: Dr. Mu-ming Poo

INSTITUTION: Columbia University
GRANT TITLE: Electrical and Chemical Modulation of Synaptic Efficacy

AWARD PERIOD: July 1, 1993 - June 30, 1996

OBJECTIVE: To investigate how electric currents associated with synaptic activity and chemical factors released by the pre- and postsynaptic cells affect the efficacy of synaptic transmission, in order to understand the plasticity of synaptic functions at single neuron level.

APPROACH: Monolayer cultures of dissociated neurons from rat hippocampi or of neurons and myocytes from *Xenopus* embryos are prepared, the synaptic functions are assayed by patch-clamp whole-cell recording and fluorescence imaging of intracellular calcium. Electrical activity is induced in the neuronal network by extracellular or intracellular stimuli and chemical factors are bath- or focally-applied to the synapses.

ACCOPLISHMENTS (last 12 months): The main finding during the past year is the our discovery of separate and synergistic actions of two neurotrophic factors, namely, brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF) at developing neuromuscular junctions in *Xenopus* nerve-muscle cultures. We found that application of these two factors resulted in an increase in the frequency of spontaneous synaptic currents and in the amplitude of nerve-evoked synaptic currents. Analyses of the amplitude and time course of the spontaneous synaptic currents suggest that these effects are due to elevation of presynaptic transmitter release. The actions of these two factors on the transmitter secretion process, however, are distinctly different. Fura-2 Ca^{2+} imaging showed that an increase in presynaptic cytosolic Ca^{2+} ($[Ca^{2+}]_i$) accompanied the synaptic potentiation by BDNF, while no change in $[Ca^{2+}]_i$ was observed during synaptic potentiation by CNTF. Removing external Ca^{2+} also abolished the potentiating effect of BDNF but did not influence the CNTF effect. Moreover, the two factors exerted different effects on the short-term synaptic plasticity: Paired pulse facilitation (PPF) normally found at these synapses was reduced by BDNF, but unaffected by CNTF; CNTF, but not BDNF, reduced the extent of synaptic depression during high frequency tetanic stimulation. Finally, the potentiation effect of BDNF and CNTF on spontaneous transmitter release was additive when both factors were applied together to the synapse at saturating concentrations (100 ng/ml), and was highly synergistic when low doses (1 and 10 ng/ml) of both factors were used. These results suggest that, due to their differential effects on the secretory machinery, BDNF and CNTF may act cooperatively in modulating the development and functioning of synapses.

SIGNIFICANCE: The results obtained in the present project provided new information concerning the cellular and molecular mechanisms of synaptic modulation, which are of

crucial importance for the understanding the computational properties of neuronal network at single neuron level. Of particular importance is our recent demonstration of the rapid actions of many neurotrophic factors in synaptic modulation. These neuropeptides are likely to be secreted by either pre- or postsynaptic cells in response to synaptic activity, thus may serve as an activity-dependent autocrine or retrograde factors in synapse modulation.

SUMMARY OF THE ENTIRE PROJECT: Work during the past three years covered three separate area of synaptic transmission: (1) Synaptic modulation by induced electrical activity, (2) synaptic modulation by neurotrophic factors, and (3) regulatory mechanisms for transmitter secretion. The results obtained are relevant to our understanding of the synaptic function and plasticity. The following description summarizes our major findings in these areas. We have completed a detail study on the effect of postsynaptic depolarizations on the depression of neuromuscular synapses. We have shown conclusively that postsynaptic elevation of calcium in both necessary and sufficient in inducing a retrograde modulation of the presynaptic transmitter secretion, leading to long-term synaptic depression. Substantial progress has also been made on the role of neurotrophins in synaptic modulation. We found marked potentiation of synaptic currents in both Xenopus nerve-muscle cultures and in hippocampal cultures by neurotrophin NT-3 and brain derived neurotrophic factor (BDNF) within minutes after application of the factor. Rapid potentiation of neuromuscular synapses by ciliary neurotrophic factor (CNTF) was also found. The synaptic action of CNTF apparently requires first a retrograde signaling to the cell body, while that of BDNF does not. Moreover, these two family of factors exert synergistic action . We have also examined the role of actin filaments in the regulation of presynaptic transmitter secretion by the use of cytochalasins, and found that actin filaments imposes a barrier for mobilization of transmitter supply, an effect that can be revealed under conditions of high demand of transmitters. The functional roles of three different synaptic vesicle associated proteins, synapsin, synaptophysin and synaptotagmin, have been determined at the developing neuromuscular synapses.

PUBLICATIONS AND ABSTRACTS (last 12 months):

1. Stoop, R., and M-m. Poo (1995) Potentiation of transmitter release by ciliary neurotrophic factor requires somatic signaling. **Science** 267: 695-699.

2. Wang, X. Zhang, J. and M-m. Poo. Effects of cytochalasin treatment on short-term synaptic plasticity at developing neuromuscular junctions in frogs. **J. Physiol. (Lond.)** 491.1: 187-195 (1996)

3. Stoop, R. and M-m. Poo (1996) Synaptic potentiation by neurotrophic factors: differential and synergistic actions of BDNF and CNTF. **J. Neurosci.** 16: 3256-3264.

Electrical and Chemical Modulation of Synaptic Efficacy (Grant #: N00014-90-J-1865, R & T Project #: 3414029)

PI: Mu-ming Poo, Columbia University

OBJECTIVE

* To investigate the effect of electric currents and chemical factors on the efficacy of synaptic transmission

* To understand the plasticity of synaptic functions at single neuron level

ACCOMPLISHMENTS

* Completed a detail study on the effect of postsynaptic depolarizations on the depression of neuromuscular synapses, and the involvement of retrograde signaling

* Discovered rapid acute potentiating action of brain derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF) on the hippocampal and spinal synapses and their synergistic actions

* Discovered that actin filaments imposes a barrier for mobilization of transmitter supply, an effect that can only be revealed under conditions of high demand of transmitters.

* Obtained evidence for functional significance of two synaptic vesicle associated proteins, synapsin IIa and synaptophysin.

SIGNIFICANCE

* Elucidation of cellular and molecular mechanisms of synaptic modulation, which are of crucial for understanding the computational properties of neuronal network at single neuron level

* Development of novel concepts on the synaptic action of neurotrophic factors in the brain